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COMPARATIVE EFFECTS OF GROWTH INHIBITORS ON STEROL METABOLISM IN THE NEMATODE *CAENORHABDITIS ELEGANS*

RUBEN LOZANO*†, DAVID J. CHITWOOD, WILLIAM R. LUSBY, MALCOLM J. THOMPSON, JAMES A. SVOBODA and GLENN W. PATTERSON*

Insect Physiology Laboratory, ARS, USDA, Beltsville, MD 20705 and *Department of Botany, University of Maryland, College Park, MD 20742, USA.
Telephone: (301) 344-2389

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Abstract—1. An analogous series of dimethylalkyl compounds, consisting of four amines, an amide, and a phosphonate ester, inhibited motility and reproduction of the nematode *Caenorhabditis elegans*.

2. Dimethylamines with straight-chain lengths of 12, 14, or 16 carbon atoms were equally active nematicides, causing greater than 80% population growth inhibition at a concentration of 25 ppm.

3. The C_{12} straight-chain amine and its corresponding amide produced similar inhibition and were much more potent than either the corresponding C_{12} phosphonate or a C_{12} branched-chain amine.

4. Inhibition of the Δ^{24}-sterol reductase system was exhibited by all four amines, but not by the amide or phosphonate, in the following order of activity: C_{12} branched-chain amine > C_{12} straight-chain amine > C_{14} amine > C_{16} amine.

5. The C_{12} branched amine also blocked the C-24(28)-dehydrogenase system in the conversion of sitosterol to fucosterol, the initial step in sitosterol dealkylation.

INTRODUCTION

Research of model compounds as potential agricultural pesticides has revealed interesting biological activities exhibited by a number of straight-chain and branched alkyl amines and amides when exposed to a wide variety of organisms. These include antimicrobial activity against mastitic bacteria of cattle (Culler *et al.*, 1979, 1980), inhibition of cellulose digestion and volatile fatty acid production by ruminal micro-organisms (Baldwin *et al.*, 1981, 1982), and toxicity towards the rabbit ear mite and psoroptic scabies mites of livestock (Fisher *et al.*, 1979; Wright *et al.*, 1979, 1980). Several alkyl amines and amides were demonstrably nematicidal towards plant-parasitic *Meloidogyne incognita* and saprophytic *Panagrellus redivivus* (Feldmesser *et al.*, 1976), the pine wood nematode *Bursaphelenchus xylophilus* (Nagase *et al.*, 1982, 1983), and animal-parasitic *Ostertagia ostertagi* (Douvres *et al.*, 1980). Their lethal effects in *O. ostertagi* occurred at the time of molt. An alkyl phosphonate ester was also highly active against the first two species (Feldmesser *et al.*, 1983). The mode of action of these chemicals in nematodes is unknown. A steroidal amine strongly interfered with the reproduction and sterol metabolism of *Caenorhabditis elegans* (Chitwood *et al.*, 1984).

The biochemical effects of these inhibitory amines and amides on other animals has been investigated. Their interference with development, molting, and metamorphosis in insects was associated with the disruption of ecdysteroid metabolism (Marks *et al.*, 1978; Svoboda *et al.*, 1978; Thompson *et al.*, 1978). In some cases, they blocked conversion of phytosterols to cholesterol in insects (Robbins *et al.*, 1975; Cohen *et al.*, 1983). Certain amines inhibited cholesterol biosynthesis in rats (Svoboda *et al.*, 1977) and chickens (Cecil *et al.*, 1981). In all cases, the hypocholesterolemic effect was mediated through inhibition of the Δ^{24}-sterol reductase enzyme system as evidenced by an accumulation of desmosterol.

Several of these inhibitory compounds (Table 1), selected for their structural relationship, were tested against *C. elegans* in order to compare their ability to inhibit growth and development of this free-living nematode, to compare their effects on sterol metabolism, notably sitosterol dealkylation and conversion to cholesterol and other metabolites, and to determine whether Δ^{24}-sterol reductase is an inhibitory site as it is in certain other species.

MATERIALS AND METHODS

Dietary sterol and inhibitors

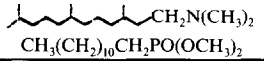
Nonradiolabeled sitosterol (24α-ethylcholest-5-en-3β-ol) was prepared from stigmasterol (Steele and Mosettig, 1963) and contained about 1.5% campesterol (24α-methylcholest-5-en-3β-ol) as an impurity by gas–liquid chromatography (GLC). [4-^{14}C]Sitosterol (Amersham Corp., Arlington Heights, Il) possessed a radiochemical purity greater than 99% by thin-layer chromatography (TLC) and GLC, and was used at a specific activity of 1000 dpm/μg (187 μCi/mmol). The tertiary alkyl amines and amide (Table 1) were prepared as described previously (Robbins *et al.*, 1975), as was the phosphonate ester (Feldmesser *et al.*, 1983).

Culture methods

Caenorhabditis elegans was cultured axenically at 22°C in an aqueous medium. The basal portion (in distilled water)

†Address for correspondence: Insect Physiology Laboratory, Bldg. 467, BARC-East, Beltsville, MD 20705, USA.

Table 1. Compounds tested against *C. elegans*

Compound	Structure
I. *N,N*-Dimethyldodecanamine	$CH_3(CH_2)_{10}CH_2N(CH_3)_2$
II. *N,N*-Dimethyltetradecanamine	$CH_3(CH_2)_{12}CH_2N(CH_3)_2$
III. *N,N*-Dimethylhexadecanamine	$CH_3(CH_2)_{14}CH_2N(CH_3)_2$
IV. *N,N*-Dimethyldodecanamide	$CH_3(CH_2)_{10}CON(CH_3)_2$
V. *N,N*-Dimethyl-3,7,11-trimethyldodecanamine	
VI. Dimethyl-1-dodecanephosphonate	$CH_3(CH_2)_{10}CH_2PO(OCH_3)_2$

consisted of yeast extract, soy peptone, dextrose, and casein hydrolyzate in final media concentrations of 30, 30, 10, and 10 g/l, respectively. All components except soy peptone were previously extracted with $CHCl_3$/MeOH (2:1) to remove endogenous sterol. A concentrated stock solution (10× the final media concentration) containing sitosterol, Tween 80, and dichloromethane-extracted hemoglobin (in final media concentrations of 25 mg/l, 1.25 ml/l, and 0.5 g/l, respectively was prepared as follows. Tween 80 was added to the sterol dissolved in benzene, solvent was removed by evaporation under N_2, warm distilled water was added slowly to the sterol–Tween mixture with vigorous agitation, and hemoglobin was dissolved in the cooled solution while stirring vigorously. One liter of medium was prepared by combining 100 ml of this stock solution with 900 ml of the basal portion. Each inhibitory test compound was simultaneously dissolved with the sterol. The solution was finally filter-sterilized and added to the autoclaved basal medium. To determine lethal or growth-inhibitory effects, a wide range of concentrations (2–1000 ppm) of the compounds was incorporated into culture media which were subsequently inoculated with *C. elegans*; the populations were examined microscopically at frequent intervals. Initial populations in inoculated culture media included 100–200 adult and juvenile nematodes per ml plus eggs. Living nematodes were isolated from logarithmic phase cultures (*ca* 16 days) by centrifugation and sucrose flotation (Fletcher and Krusberg, 1973).

Sterol analysis

Total lipids from lyophilized nematodes were extracted essentially according to Folch *et al.* (1957) by homogenization with 4 volumes of $CHCl_3$/MeOH (2:1) using a Ten-Broeck tissue grinder. Neutral lipids were separated from polar lipids by elution of the total lipid extract with $CHCl_3$ through a column of silica gel 60 (E. Merck, Darmstadt, FRG). The neutral lipid fraction was then saponified in 5% KOH in MeOH (w/v) for 4 hr at 70°C, extracted with hexane, and chromatographed on a 7.0 g column of silica gel 60 eluted with ether graded into hexane (respectively, 20 ml each of 10, 12, 14, 16, 17, 20, and 25% ether in hexane, 50 ml of 30%, and 20 ml ether). Fractions were monitored by TLC using Anasil H plates (Analabs, North Haven, CT) developed in two solvent systems: S_1, benzene/diethyl ether (80:20); S_2, hexane/diethyl ether/acetic acid (80:20:1). Compounds were detected by spraying with 50% H_2SO_4 and charring at 110°C. The column elution resulted in the isolation of separate 4-methylsterol (eluted with 17 and 20% ether in hexane) (R_f: S_1, 0.30; S_2, 0.15) and 4-desmethylsterol (25 and 30% ether in hexane) (R_f: S_1, 0.21; S_2, 0.09) fractions which were quantitatively and qualitatively analyzed by GLC. Sterols were fractionated further by: acetylation with pyridine–acetic anhydride (2:1) overnight at room temperature; purification of the steryl acetates on silica gel columns (R_f of cholesteryl acetate on TLC: S_1, 0.64; S_2, 0.50); argentation column chromatography (Vroman and Cohen, 1967) of the purified steryl acetates on 20% $AgNO_3$-impregnated Unisil eluted with 1% increments of ether/hexane; and GLC of the separated acetates. Argentation TLC of column fractions was performed on 10% $AgNO_3$-Anasil plates developed in benzene/hexane (2:1). Identifications were confirmed by gas chromatography–mass spectrometry (GC–MS) and ultraviolet (u.v.) spectroscopy. Column fractions were also monitored by measuring radioactivity of aliquots. Compounds trapped from the GLC effluent were counted to determine specific activities.

Instrumentation

GLC was carried out on a Varian 3700 gas chromatograph equipped with a flame ionization detector for analytical performance and a thermal conductivity detector for preparative trapping of GLC effluent. Analyses were made using three chromatographic systems: 2.0% SE-30 at 240°C, or 2.0% OV-17 at 250°C, each packed in a coiled glass column (2 m × 2 mm i.d.) with He carrier gas at 35 ml/min; and a J&W fused silica capillary column (13.8 m × 0.25 mm i.d.) with a 0.25 μm film of DB-1 (bonded methyl silcone) at 240°C, He at 40 cm/s linear velocity, 50:1 split ratio. Retention times and peak areas were measured by a Shimadzu C-R1B Chromatopac data processor. GC–MS was performed on a Finnigan model 4510 instrument equipped with a 15 m × 0.32 mm DB-1 capillary column (0.25 μm film) and interfaced with an Incos Data System. Ultraviolet spectra of steryl acetates in hexane were obtained with a Perkin–Elmer 559 spectrophotometer. Radioactivity was measured with a Packard TRI-CARB 460 CD Liquid Scintillation System; samples were dissolved in a toluene-based cocktail containing 0.5% PPO and 0.03% dimethyl POPOP.

RESULTS

Growth inhibition

Each of the six test compounds was inhibitory towards *C. elegans* as motility and reproduction of treated nematodes decreased with increasing concentrations of each chemical. Paralysis of the entire

Table 2. Range of concentrations (in ppm) of test compounds resulting in 100% paralysis of *C. elegans* populations after various exposure periods*

Time of exposure to test compound	C_{12} amine	C_{14} amine	C_{16} amine	C_{12} amide	Branched C_{12} amine	C_{12} phosphonate
Immediate	50–80	55–60	60–70	40–45	>500	>1000
2 days	35–40	55–60	40–45	40–45	>500	>1000
3 days	30–35	40–45	30–35	40–45	>500	>1000
1 week	30–35	40–45	30–35	40–45	400–500	>1000
2 weeks	30–35	40–45	30–35	40–45	200–300	400–600
3 weeks	30–35	40–45	30–35	40–45	200–300	200–400
4 weeks	30–35	40–45	30–35	40–45	200–300	200–400

*Media were supplemented with 25 ppm sitosterol.

Table 3. Effects of inhibitory test compounds on population growth and lipid and sterol content of *C. elegans**

Inhibitor	Inhibitor concentration (ppm)	Culture density† (nematodes/ml)	Dry weight (g/l)	Lipid content (% of dry weight)	Sterol content‡ (% of dry weight)	Sterol content‡ (pg per nematode)
Control		79.3×10^3	1.61	18.3	0.09	17.5
C_{12} amine	25	13.5×10^3	0.34	20.2	0.10	24.7
C_{14} amine	25	10.4×10^3	0.23	19.4	0.09	19.9
C_{16} amine	25	11.9×10^3	0.32	21.5	0.04	11.8
C_{12} amide	35	14.5×10^3	0.61	19.0	0.11	32.9
Branched C_{12} amine	25	45.0×10^3	1.25	18.9	0.02	6.6
C_{12} phosphonate	50	35.0×10^3	0.89	17.5	0.06	16.4

*Media were supplemented with 25 ppm sitosterol.
†Only juveniles and adults were counted.
‡Determined by GLC quantitation.

population occurred at some inhibitor concentration depending upon the time of exposure (Table 2). The three straight-chain amines and the amide were almost equally active in producing 100% paralysis of *C. elegans* after 1 week at concentrations of 30–45 ppm. The branched-chain amine and the phosphonate ester were approximately an order of magnitude less potent, requiring over 400 ppm to produce similar effects.

To examine their effects on sterol metabolism in *C. elegans*, sublethal concentrations of each inhibitor were arbitrarily chosen for media incorporation. Various growth and extraction data were compiled from the harvested cultures (Table 3). As compared to the control, population density values revealed that: at 25 ppm, the C_{12}, C_{14}, and C_{16} straight-chain amines and the C_{12} branched-chain amine caused 83, 87, 85, and 43% reductions in population, respectively; the C_{12} amide resulted in 82% reduction at 35 ppm; and the C_{12} phosphonate caused a 56% population decrease at 50 ppm. These values, as well as the dry weight measurements, reflected the general degree of activity observed in the previous toxicity studies: the three straight-chain amines and the amide were similarly effective as inhibitors of reproduction and much more so than the branched-chain amine or the phosphonate. Total lipid content of inhibitor-treated cultures ranged from 17.5 to 21.5% of the dry weight as compared to 18.3% in the control culture. Greater variation in sterol content of treated cultures was seen; especially low values were obtained with the branched-chain amine.

Sterol analyses

In agreement with our previous results (Chitwood *et al.*, 1983, 1984), in the absence of an inhibitor (Table 4, control), *C. elegans* dealkylated sitosterol and produced cholesterol, 7-dehydrocholesterol, lathosterol, 4α-methylcholest-7-enol, and 4α-methylcholest-8(14)-enol (possibly in that sequence), with 7-dehydrocholesterol as the predominant sterol. The sterol compositions of inhibitor-treated *C. elegans* cultures supplemented with sitosterol are listed in Table 4. The relative percentages of Δ^{24}-sterols were particularly noted: in the absence of any inhibitor they were not detected; relatively small amounts were found in the presence of either the amide or phosphonate; but in the presence of the C_{12}, C_{14}, and C_{16}

Table 4. Relative percentages of total sterols from *C. elegans* propagated with sitosterol plus different inhibitors*

	% of total sterol						
	Inhibitor and concentration						
Sterol†	Control	C_{12} amine, 25 ppm	C_{14} amine, 25 ppm	C_{16} amine, 25 ppm	C_{12} amide, 35 ppm	Branched C_{12} amine, 25 ppm	C_{12} phosphonate, 50 ppm
7-Dehydrocholesterol	51.0	23.1	33.7	29.0	47.0	3.7	53.3
Cholesta-5,7,24-trienol	‡	14.8	9.7	2.4	‡	7.4	2.2
Sitosterol	18.4	19.6	16.8	20.0	20.1	35.7	13.1
Fucosterol	‡	0.4	2.7	1.7	1.1	3.8	1.0
4α-Methylcholest-8(14)-enol	10.5	4.1	5.3	11.4	6.7	2.8	11.6
4α-Methylcholesta-8(14),24-dienol	‡	2.2	1.0	1.4	‡	9.1	‡
Cholesterol	9.2	9.0	9.3	8.5	9.0	2.6	6.6
Desmosterol	‡	4.7	‡	0.8	‡	11.8	‡
Lathosterol	6.8	7.7	6.1	7.2	6.0	2.0	4.5
Cholesta-7,24-dienol	‡	1.2	1.2	1.1	‡	6.9	‡
Cholesta-8,24-dienol	‡	0.1	‡	‡	‡	3.3	‡
Cholesta-5,7,9(11)-trienol	3.3	0.7	3.8	11.0	6.4	0.5	7.0
Cholesta-5,7,9(11),24-tetraenol	‡	1.4	1.6	‡	‡	‡	‡
Campesterol	0.4	1.5	1.3	2.2	1.0	3.6	0.3
4α-Methylcholest-7-enol	0.4	2.9	2.6	2.9	0.7	2.1	0.4
4α-Methylcholesta-7,24-dienol	‡	1.7	0.9	0.4	‡	4.5	‡
Others (unidentified)	‡	4.9	4.0	‡	2.0	0.2	‡
Total Δ^{24}-sterols	‡	26.5	17.1	7.8	1.1	46.8	3.2
Total 24-alkylsterols	18.8	21.5	20.8	23.9	21.1	43.1	14.4

*Media contained 25 ppm sitosterol (with campesterol as a slight impurity).
†Nomenclature for trivial names: cholesterol = cholest-5-en-3β-ol; lathosterol = cholest-7-en-3β-ol; desmosterol = cholesta-5,24-dien-3β-ol; fucosterol = 24-*cis*-ethylidenecholest-5-en-3β-ol.
‡Not detected.

Table 5. GLC analysis of sterols from *C. elegans*

Sterol acetate	RRT OV-17	RRT SE-30	RRT DB-1
Cholesterol	1.00	1.00	1.00
Cholesta-5,7,9(11)-trienol	1.04	0.94	0.98
7-Dehydrocholesterol	1.17	1.08	1.14
Lathosterol	1.17	1.11	1.17
Desmosterol	1.20	1.08	1.11
Cholesta-5,7,9(11),24-tetraenol	1.25	1.04	1.08
Cholesta-8,24-dienol	1.27	1.13	1.17
Campesterol	1.32	1.34	1.32
Cholesta-5,7,24-trienol	1.39	1.18	1.24
Cholesta-7,24-dienol	1.41	1.21	1.26
Sitosterol	1.62	1.60	1.62
Fucosterol	1.72	1.60	1.62
4α-Methylcholest-8(14)-enol	1.12	1.16	1.18
4α-Methylcholest-7-enol	1.30	1.28	1.32
4α-Methylcholesta-8(14),24-dienol	1.36	1.25	1.28
4α-Methylcholesta-7,24-dienol	1.59	1.40	1.44

RTT = Retention time of sterols as acetate derivatives relative to cholesteryl acetate. Chromatographic conditions are described in Materials and Methods.

straight- and branched-chain amines, they comprised relatively significant proportions of the total sterol. Identification of sterols from *C. elegans* was made on the basis of their GLC RRT values, both as free sterols and as steryl acetates (Table 5), and GC–MS analyses. All data were in agreement with those of authentic standards, with the exception of the following, for which no reference compounds were available: cholesta-7,24-dienol, cholesta-8,24-dienol, cholesta-5,7,9(11),24-tetraenol, and the pair of 4-methylcholestadienols. Nevertheless, the experimental RRTs of these five sterols closely approximated their calculated values, and the proposed structures were verified by expected fragmentation patterns from GC–MS. Mass spectral data for six related sterols from *C. elegans* are compared in Table 6; the sterols were analyzed as acetates of 4-methylcholest-8(14)-enol, 4-methylcholest-7-enol, 4-methylcholesta-8(14),24-dienol, 4-methylcholesta-7,24-dienol, cholesta-8,24-dienol, and cholesta-7,24-dienol. Identification of the four 4-methylsterols has been reported previously (Chitwood *et al.*, 1983) including mass spectra of the free sterol forms. Sterol identification was also corroborated by their comparable migration with standards in argentation chromatography (column and TLC). Typical u.v. spectra of $\Delta^{5,7}$-dienes (λ_{max} 270, 281, 293 nm, and an inflection at 262) were exhibited by the acetates of 7-dehydrocholesterol and cholesta-5,7,24-trienol. Ultraviolet absorption characteristic of a $\Delta^{5,7,9(11)}$-triene (λ_{max} 308, 322, 338 nm) was demonstrated by the acetates of cholesta-5,7,9(11)-trienol and cholesta-5,7,9(11),24-tetraenol. The sitosterol and campesterol originated from the medium.

In each case where the C_{12} and C_{14} straight-chain amines and the C_{12} amide were utilized in media supplemented with ^{14}C-sitosterol, all sterols isolated from *C. elegans* were radiolabeled with approximately the same specific activity as the dietary sterol with the exception of cholesta-8,24-dienol, of which insufficient quantity was available for an accurate determination. Since all of the sterols identified from the other inhibitor-treated cultures were identical to the labeled sterols, they were assumed to be metabolites originating from the dietary sitosterol.

DISCUSSION

Each of the six test compounds demonstrated inhibitory activity towards *C. elegans* resulting in reduction or cessation of motility and reproduction of the nematodes. Of the group, the C_{12}, C_{14}, and C_{16} straight-chain amines and the C_{12} amide were the most active, causing 100% paralysis after 1 week of exposure to a concentration of 30–45 ppm. They were much less active against *C. elegans* than other nematode species; the same or closely related inhibitors were lethal at 5–10 ppm or less in *Panagrellus redivivus* (Feldmesser *et al.*, 1976) and 1–2 ppm in *Ostertagia ostertagi* (Douvres *et al.*, 1980). In con-

Table 6. Mass spectral data for several 4-methyl and 4-desmethyl steryl acetates derived from *C. elegans*

Fragmentation*	4-CH_3-Cholestenol $\Delta^{8(14)}$ (*m/z*)	4-CH_3-Cholestenol $\Delta^{8(14)}$ (%)	4-CH_3-Cholestenol Δ^{7} (%)	4-CH_3-Cholestadienol $\Delta^{8(14),24}$ (*m/z*)	4-CH_3-Cholestadienol $\Delta^{8(14),24}$ (%)	4-CH_3-Cholestadienol $\Delta^{7,24}$ (%)	Cholestadienol $\Delta^{8(9),24}$ (*m/z*)	Cholestadienol $\Delta^{8(9),24}$ (%)	Cholestadienol $\Delta^{7,24}$ (%)
M^+	442	53	40	440	19	1	426	23	10
M^+–CH_3	427	7	3	425	6	3	411	10	12
M^+–CH_3–Ac	367	6	3	365	5	1	351	8	5
M^+–SC	329	5	2	329	1	1	315	1	3
M^+–SC–2H	327	†	†	327	2	67	313	3	100
M^+–SC–Ac	269	18	67	269	2	4	255	3	14
M^+–SC–Ac–C_2H_2	243	24	12	243	9	2	229	11	7
M^+–SC–Ac–C_3H_6	227	36	32	227	16	10	213	32	27
C_5H_9	69	58	53	69	100	100	69	100	95
C_4H_7	55	100	100	55	57	68	55	51	58

*Ac = CH_3COOH; SC = side-chain.
†Not detected.

trast, the C_{12} amide was ineffective against *Meloidogyne incognita* (Feldmesser *et al.*, 1976). The C_{12} branched-chain amide was relatively inactive towards *C. elegans* compared to its lethality at a dose of 1–2.5 ppm in *O. ostertagi*. Similarly inactive towards *C. elegans* was the C_{12} phosphonate, whereas in *P. redivivus* it was effective at 0.5–1 ppm (Feldmesser *et al.*, 1983). Thus, all of the inhibitory compounds were generally less potent against the free-living *C. elegans* than against other nematode species previously tested. Their degree of nematicidal activity is apparently species-dependent. These inhibitors are not necessarily more active against parasitic nematodes such as *Ostertagia* or *Meloidogyne*, since they were also more active against *Panagrellus*, even though both it and *Caenorhabditis* are nonparasitic genera. *C. elegans* may possess enhanced physiological defenses against toxins, e.g. greater cuticular or membrane impermeability, or more rapid enzymatic degradation. Alternatively, the differences in activity may be due to differences between the test media. The presence of a sterol in our media could have reduced the toxicity of these inhibitors towards *C. elegans* by competition at some active site of metabolism or uptake, as previous experiments with *Panagrellus* and *Meloidogyne* were direct contact tests performed in water–sand–toxicant mixtures.

No distinct relationship was ascertained between the chain-length structure (C_{12}, C_{14}, C_{16}) of the straight-chain amines and their nematicidal activity towards *C. elegans*. In previous tests against *O. ostertagi* (Douvres *et al.*, 1980), C_{18} amines permitted development of infective larvae to a small percentage of mature adults, whereas C_{12} and C_{14} amines at the same concentration were more toxic, producing even lower yields of advanced stages. A greater number of amines of varying chain lengths should be tested and more precise criteria such as an examination of different developmental stages should be utilized to establish the chain length optimal for biological activity against *C. elegans*.

The relationship between the series of four C_{12} compounds (straight-chain amine, branched-chain amine, amide, and phosphonate ester) and their nematicidal activity can be examined: the straight-chain amine and amide were similarly active and much more so than either the phosphonate or branched amine. Thus, increasing the molecular bulk of the C_{12} straight-chain dimethylamine by either lengthening the chain to 16 carbon atoms or converting the amine to an amide did not affect the nematicidal activity. However, when the bulk was increased by the insertion of three methyl groups along the chain or substitution of the amine with phosphonate, the inhibitory activity was decreased. While the latter two chemicals may be intrinsically less toxic, possibly they possess modes of action altogether different from the others. Alternatively, the increased structural bulk may have retarded the uptake of those molecules by the nematodes or else enhanced their metabolic deactivation. The inhibitors did not affect the total lipid content as a percentage of dry weight; the experimental values (17.5–21.5%) were similar to those reported elsewhere for *C. elegans*: 18.3% (Chitwood *et al.*, 1984) and 19.6% (Hutzell and Krusberg, 1982). They did produce a wide variation in sterol content; the branched amine caused a substantial decrease, perhaps by interfering with sitosterol uptake from the medium by *C. elegans*.

Sitosterol dealkylation in insects proceeds via $\Delta^{24(28)}$-sterols to Δ^{24}-sterols to cholesterol, the latter step being catalyzed by the Δ^{24}-sterol reductase enzyme system (Svoboda *et al.*, 1978). Previous treatment of sitosterol-propagated *C. elegans* with an azasteroid (25-azacoprostane) produced a significant accumulation of Δ^{24}-sterols, indicating inhibition of the Δ^{24}-sterol reductase system (Chitwood *et al.*, 1983, 1984). Similarly, in the present study, several growth inhibitors blocked this enzyme to varying degrees. Identification of the metabolites from sitosterol supports our belief, as recently postulated (Chitwood *et al.*, 1984), that the pathway of sitosterol dealkylation in *C. elegans* is very similar to that in insects (Svoboda *et al.*, 1978) involving fucosterol and desmosterol as intermediates. The C_{12} branched-chain amine was by far the most inhibitive toward the Δ^{24}-sterol reductase, resulting in an accumulation of 47% Δ^{24}-sterols. Of the straight-chain amines, Δ^{24}-sterol accumulation was greatest with the C_{12} amine and decreased as the chain length increased. The amide and the phosphonate demonstrated very little Δ^{24}-sterol reductase inhibition. Replacement of the dietary sitosterol with stigmasterol (22-*trans*-dehydrositosterol) yielded similar results (unpublished): a substantial accumulation of Δ^{24}-sterols was detected in stigmasterol-supplemented cultures treated with the C_{12} straight-chain amine (33.5% of total sterol) but not with the amide (0.4–1.2%). All six compounds tested here were less potent than 25-azacoprostane as inhibitors of the Δ^{24}-sterol reductase in *C. elegans*; Δ^{24}-sterols comprised 97% of the total sterol from nematodes treated with 5 ppm of the azasteroid.

Interestingly, the branched-chain amine caused a substantial increase in the percentage of unmetabolized dietary sitosterol, a finding not evidenced in the other treated cultures. In addition to inhibiting the Δ^{24}-sterol reductase, *N*,*N*,3,7,11-pentamethyldodecanamine apparently also blocks the initial step in sitosterol dealkylation: presumably the introduction of a $\Delta^{24(28)}$-bond to produce fucosterol. The inhibition of this step by the C_{12} branched amine but not by the straight-chain analog is likely due to the steric effect of the C-3 methyl group; the resulting amine chain bears structural resemblance to the alkylated side-chain of sitosterol or campesterol. Thus, the branched-chain amine is recognized by the C-24(28)-dehydrogenase and competes with dietary sitosterol for the binding site. Likewise, the structural resemblance of each amine chain to the Δ^{24}-sterol side-chain allows for their competitive inhibition of the Δ^{24}-sterol reductase. Substitution of the amine with an amide or phosphonate group virtually abolished the latter enzymatic inhibition. None of the test compounds seemed to affect sitosterol metabolism involving 4α-methylation, C-7 dehydrogenation (to form $\Delta^{5,7}$-sterols), or C-5 reduction (to form Δ^{7}-sterols). Although certain of the inhibitors caused dramatic decreases in the percentage of 7-dehydrocholesterol, these reductions occurred concomitantly with increases in the relative amounts of

cholesta-5,7,24-trienol due to Δ^{24}-sterol reductase inhibition. Interference with other pathways of steroid metabolism, including possible ecdysteroid biosynthesis, has not been ruled out.

The comparative biochemical action of the test compounds on the Δ^{24}-sterol reductase in other species reveals both similarities and differences. *C. elegans* was similar to two insect species, the tobacco hornworm (Robbins *et al.*, 1975) and the Indian meal moth (Cohen *et al.*, 1983), in the following ways: the amines were inhibitory but the amide was not; the C_{12} branched amine was more active than its straight-chain analog. The organisms differed in that the C_{12} straight-chain amine was more active than the C_{14} and much more active than the C_{16} in *C. elegans*, whereas a C_{15} dimethylamine was more inhibitory than the C_{12} in both insects. *C. elegans* was similar to a mammal as the Δ^{24}-sterol reductase in the rat (Svoboda *et al.*, 1977) was also blocked by the four amines, of which the branched compound was the most effective. In contrast, *C. elegans* differed from birds in that only the C_{12} branched amine and not any of the straight-chain amines decreased plasma or egg cholesterol in chickens (Cecil *et al.*, 1981).

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